Improving RNA Modification Mapping Sequence Coverage by LC-MS through a Nonspecific RNase U2-E49A Mutant

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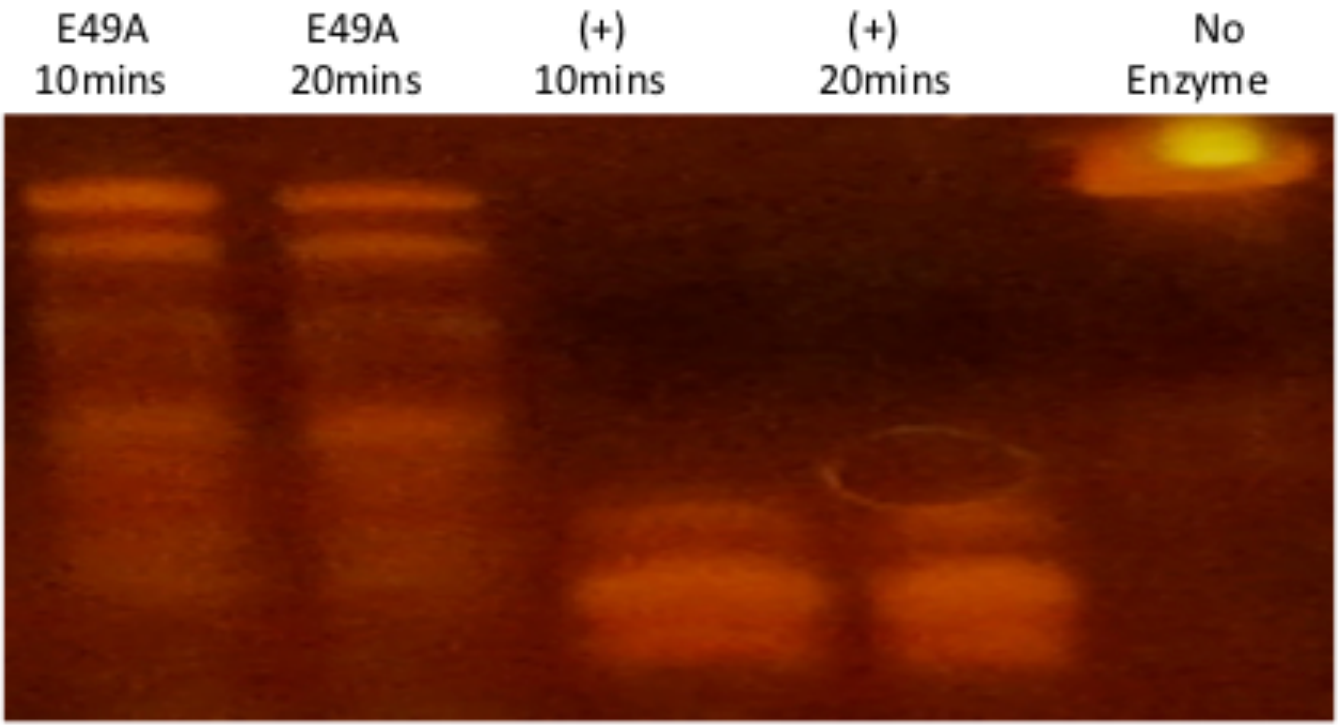
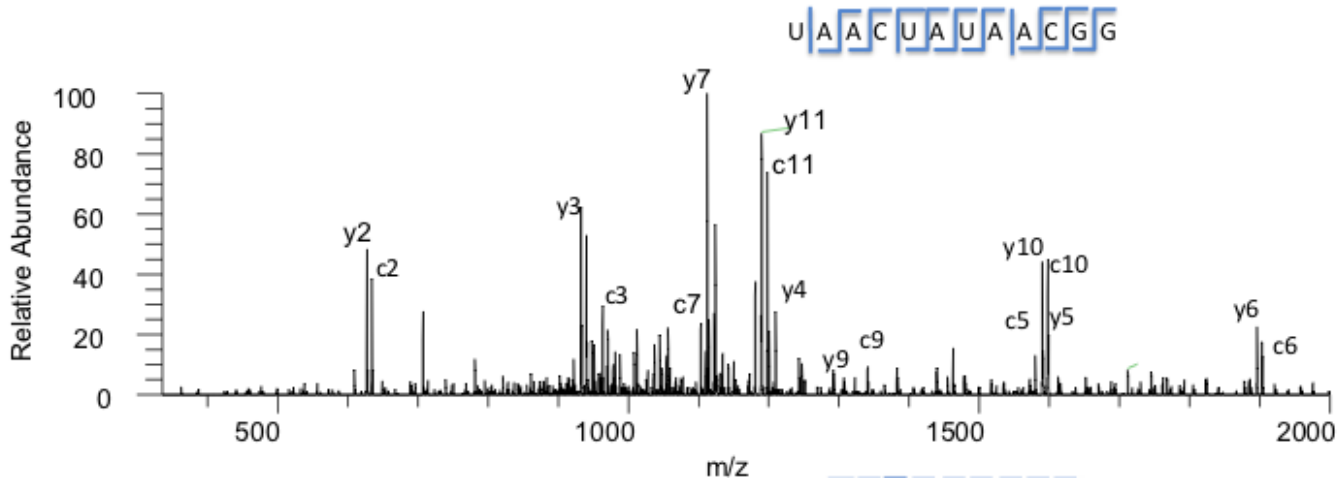
SUPPLEMENTAL INFORMATION

Supplemental Figures S1-S22

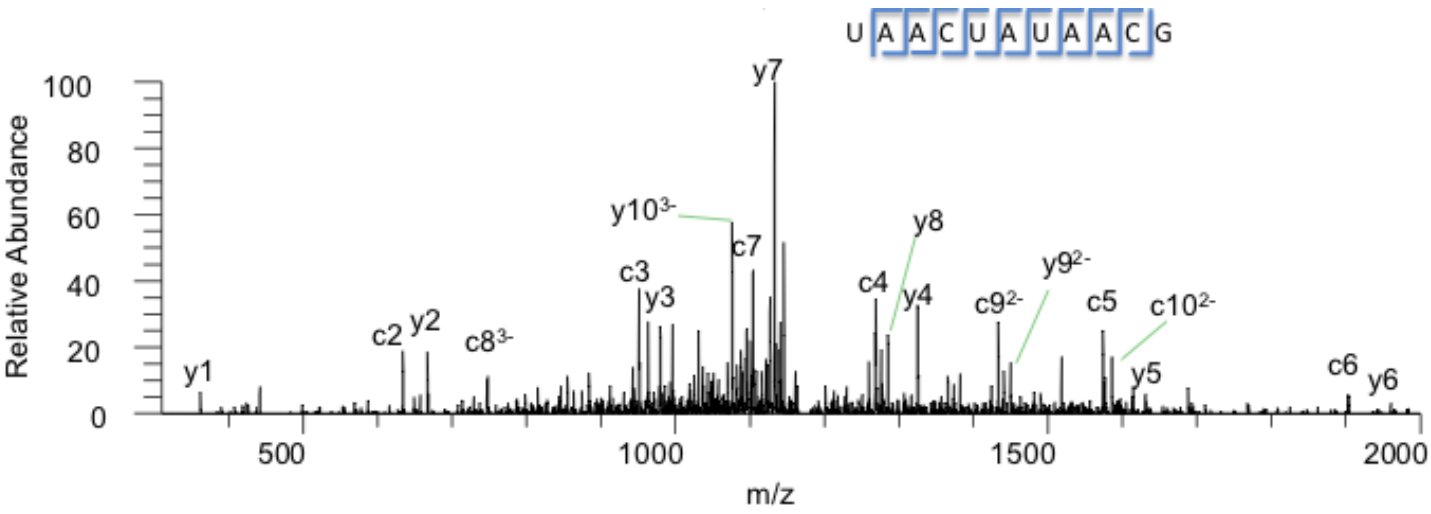
Supplemental Table S1



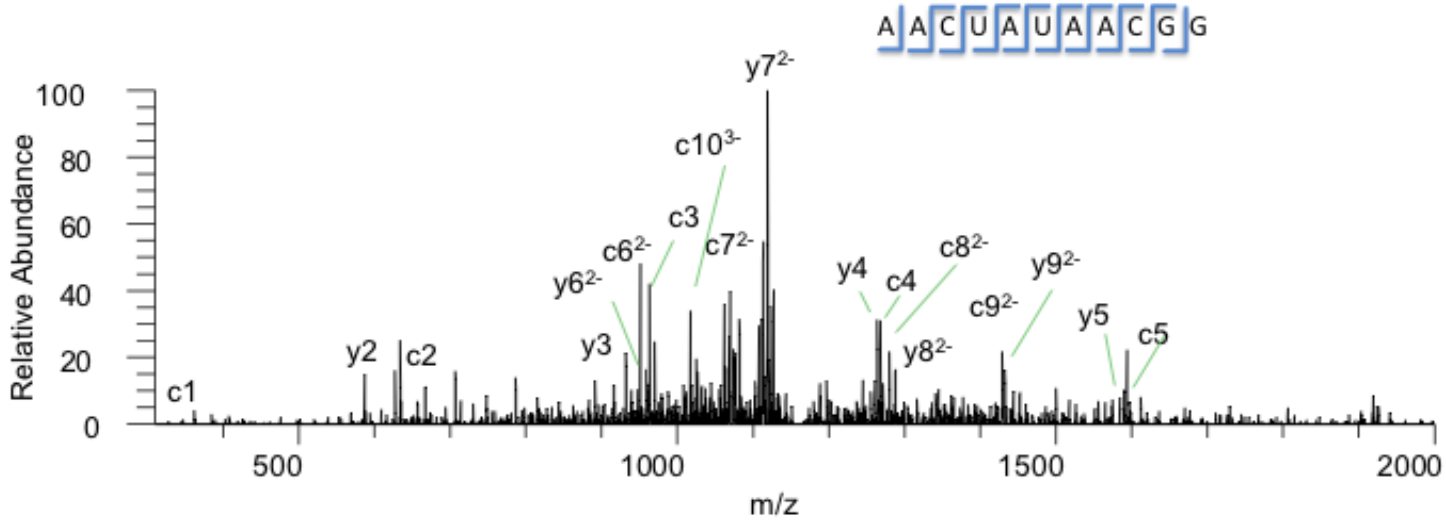
Supplemental Figure S1. Activity of E49A RNase U2 mutant compared to codon-optimized RNase U2. 1 ng of enzyme was incubated with 3 µg *Saccharomyces cerevisiae* $tRNA^{Phe}$ in 50 mM ammonium acetate, pH 4.5, at room temperature for 10 and 20 min. Samples were loaded onto a 15% TBE-urea polyacrylamide gel and run for 1 h at 180 V. Enzyme activity was determined by quantifying the amount of undigested tRNA after incubation. The E49A mutant was found to be ~15% less active than codon optimized RNase U2.



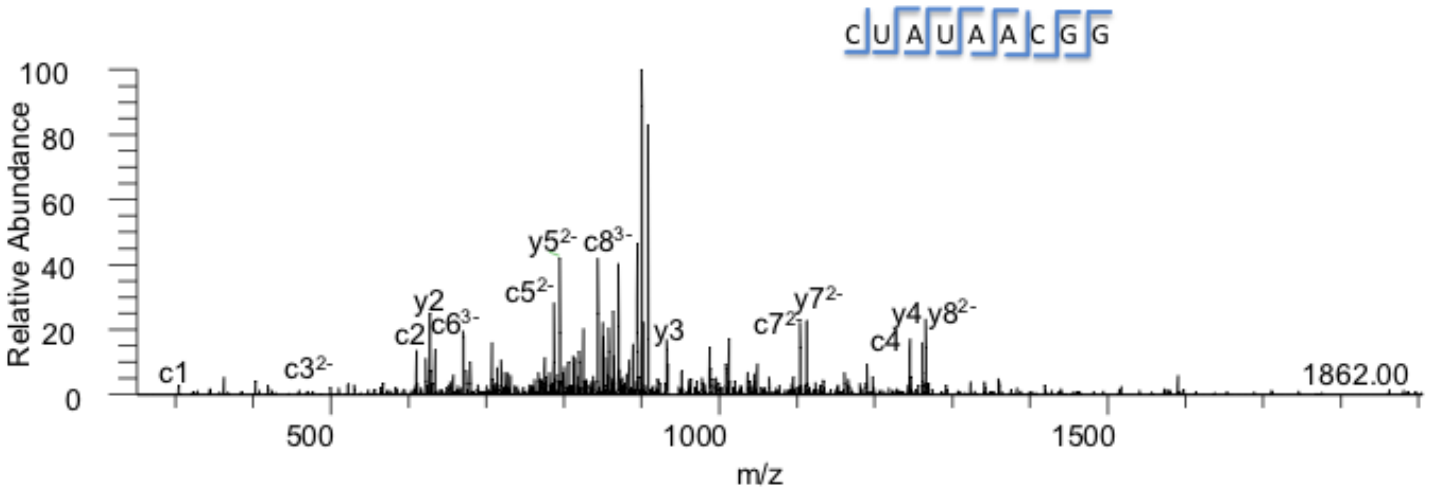
Supplemental Figure S2. CID mass spectrum with product ion assignments of the undigested synthetic oligonucleotide 5'-UAACUAUAACGG-3' upon incubation with the E49A mutant for 30 minutes.



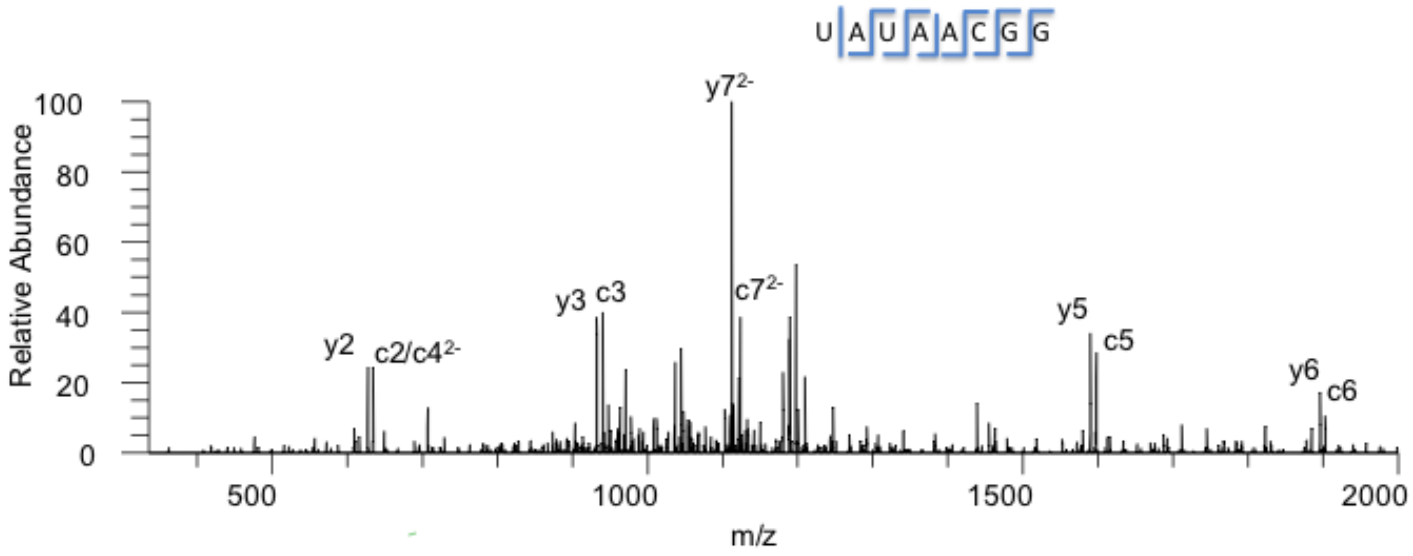
Supplemental Figure S3. CID mass spectrum with product ion assignments of the 5'-UAACUAUAACG-3' digestion product upon incubation with the E49A mutant for 30 minutes



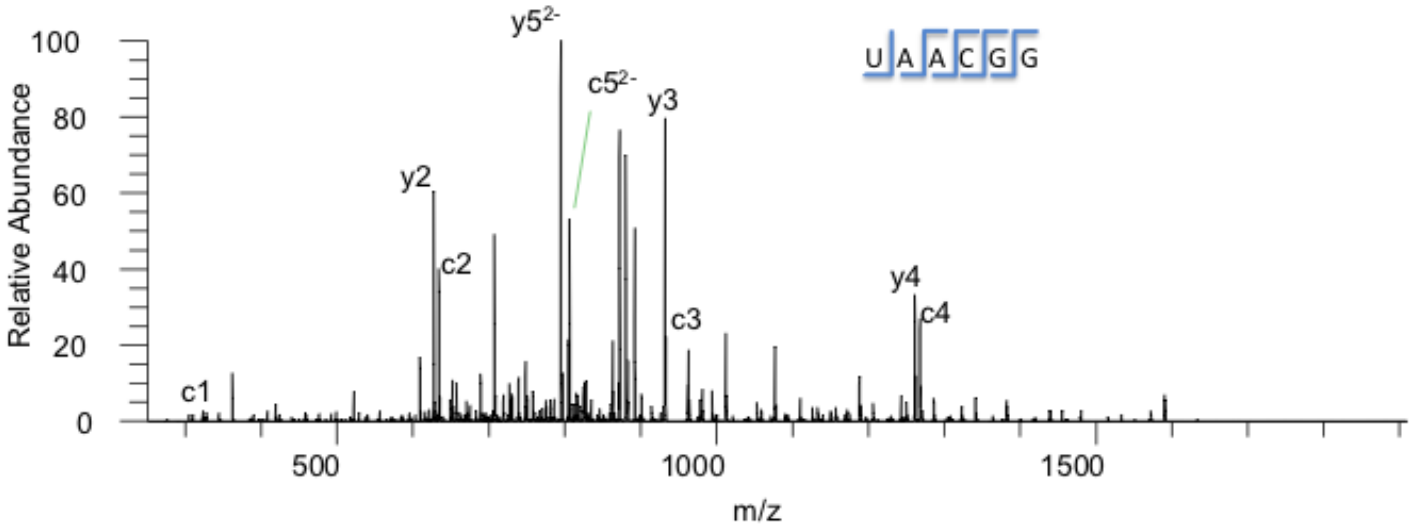
Supplemental Figure S4. CID mass spectrum with product ion assignments of the 5'-AACUAUAACGG-3' digestion product upon incubation with the E49A mutant for 30 minutes



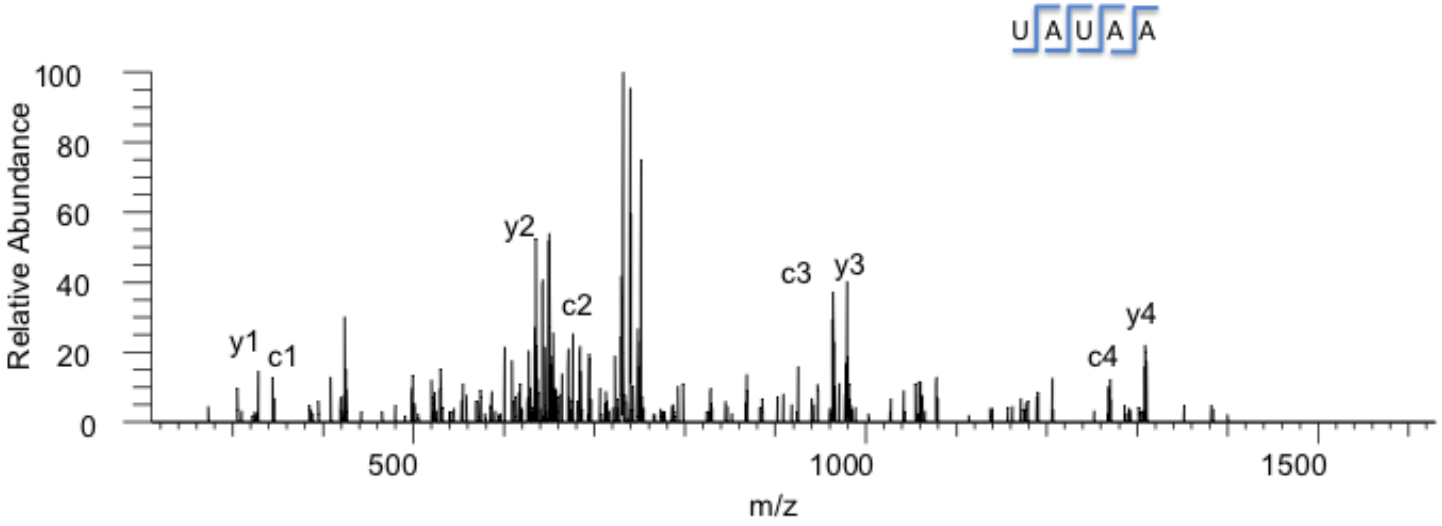
Supplemental Figure S5. CID mass spectrum with product ion assignments of the 5’-CUAUAACGG-3’ digestion product upon incubation with the E49A mutant for 30 minutes



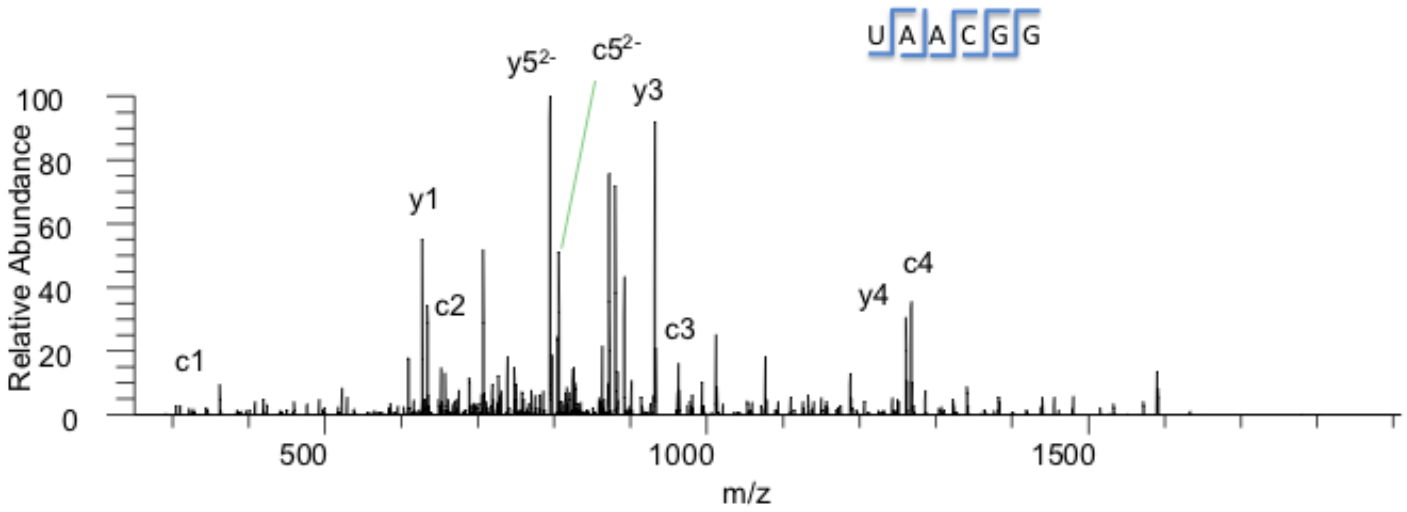
Supplemental Figure S6. CID mass spectrum with product ion assignments of the 5’-UAUAACGG-3’ digestion product upon incubation with the E49A mutant for 30 minutes



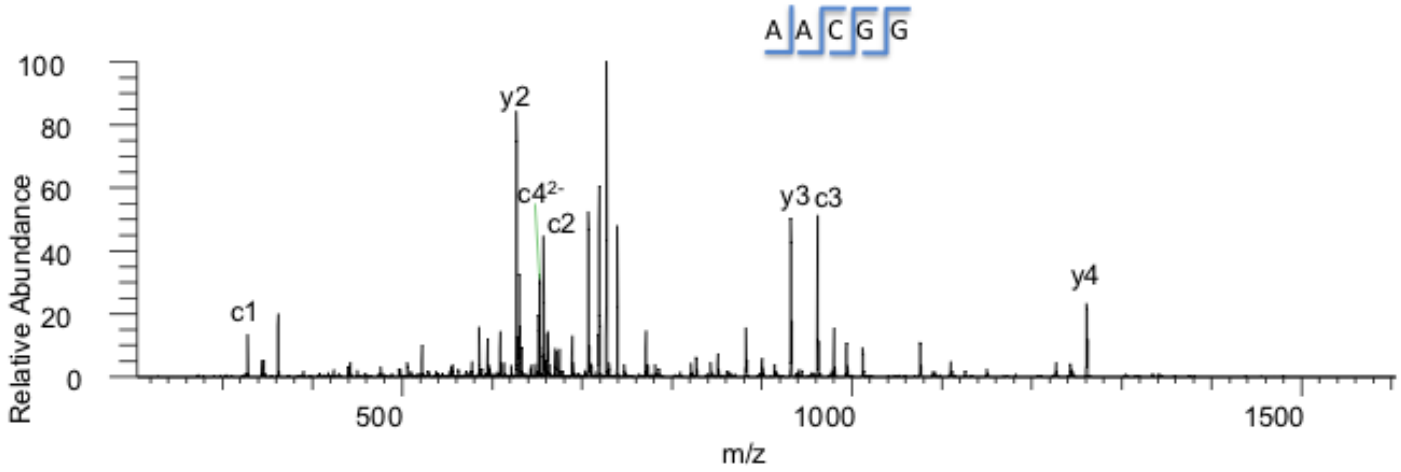
Supplemental Figure S7. CID mass spectrum with product ion assignments of the 5’-UAACG-3’ digestion product upon incubation with the E49A mutant for 30 minutes



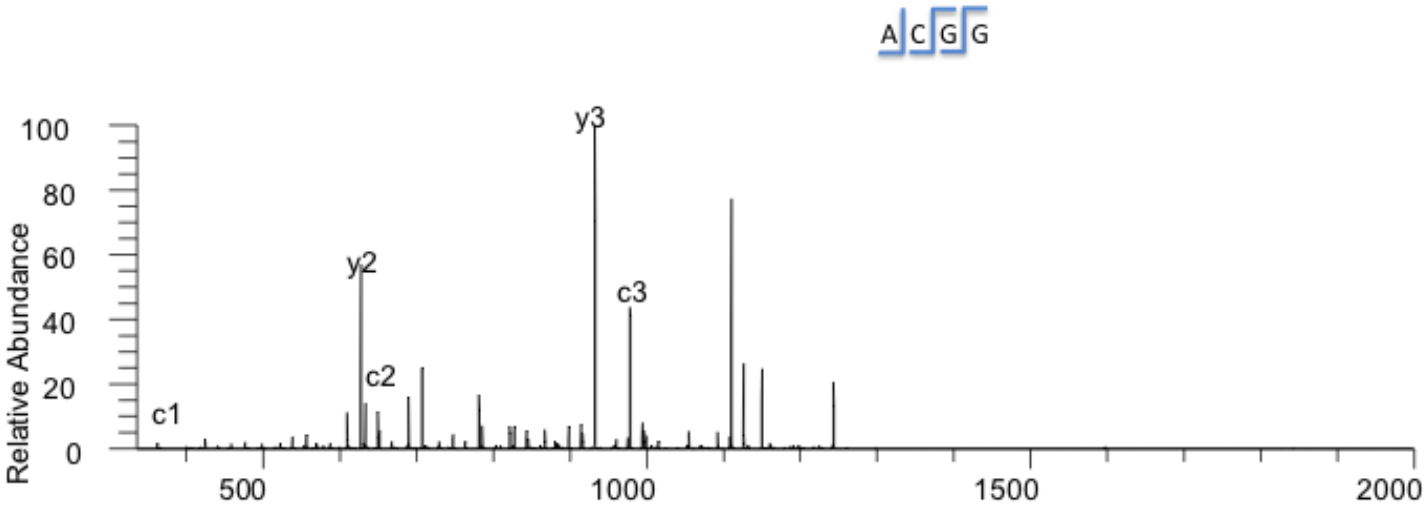
Supplemental Figure S8. CID mass spectrum with product ion assignments of the 5'-UAUAA-3' digestion product upon incubation with the E49A mutant for 30 minutes



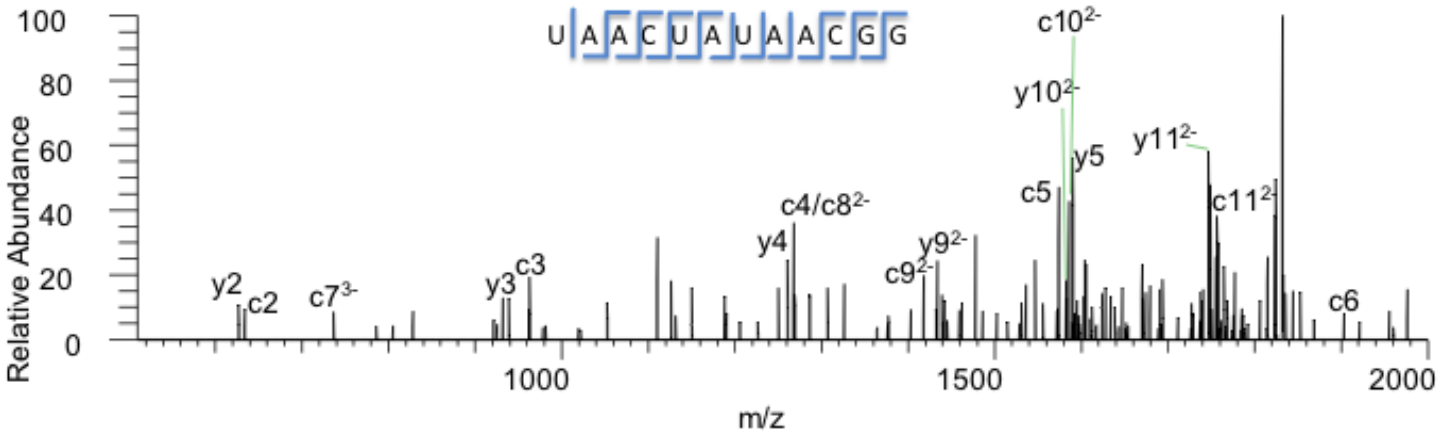
Supplemental Figure S9. CID mass spectrum with product ion assignments of the 5'-UAACGG-3' digestion product upon incubation with the E49A mutant for 30 minutes



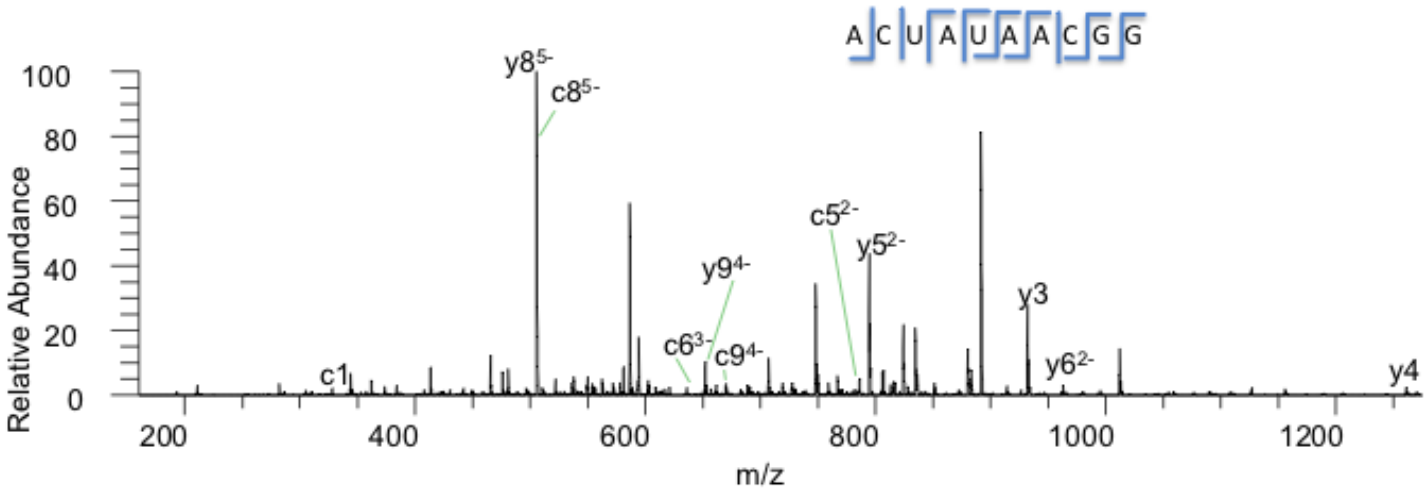
Supplemental Figure S10. CID mass spectrum with product ion assignments of the 5'-AACGG-3' digestion product upon incubation with the E49A mutant for 30 minutes



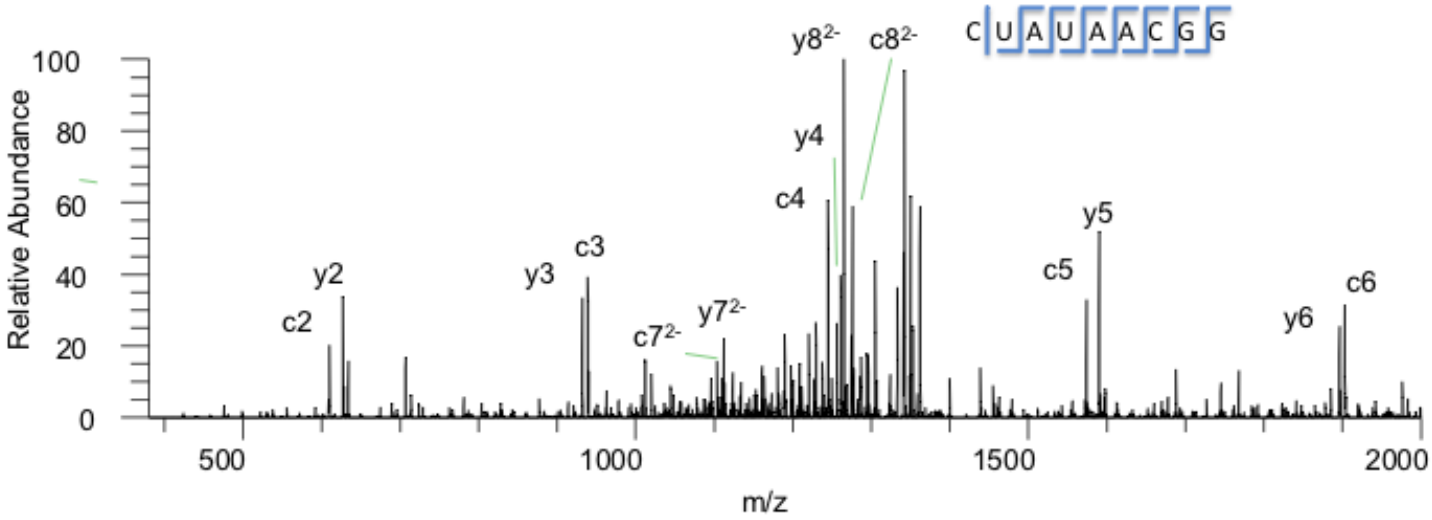
Supplemental Figure S11. CID mass spectrum with product ion assignments of the 5’-ACGG-3’ digestion product upon incubation with the E49A mutant for 30 minutes



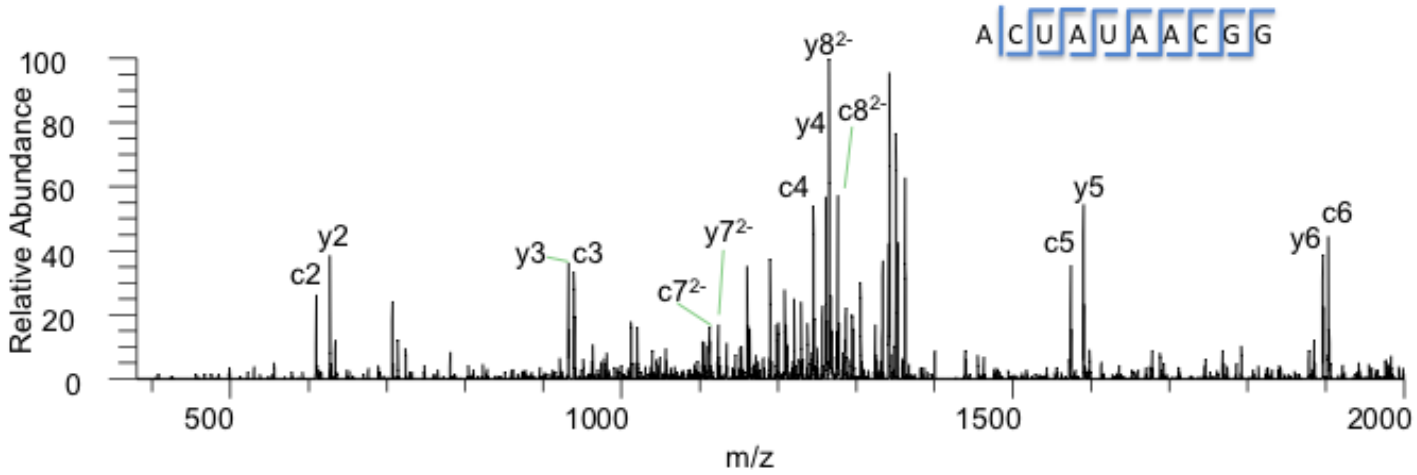
Supplemental Figure S12. CID mass spectrum with product ion assignments of the undigested synthetic oligonucleotide 5'-UAACUAUAACGG-3' upon incubation with codon-optimized RNase U2 for 30 minutes



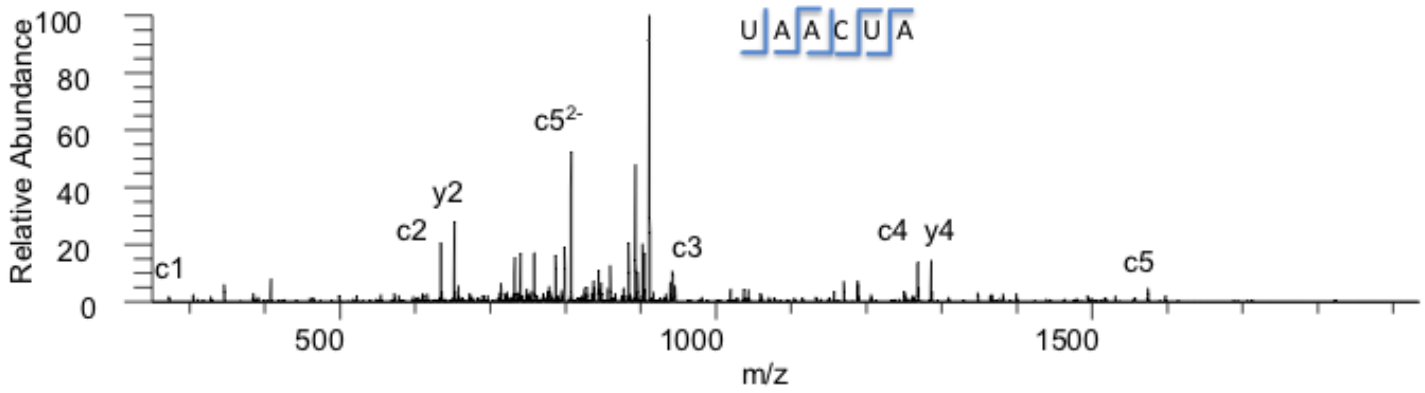
Supplemental Figure S13. CID mass spectrum with product ion assignments of the 5'-ACUAUAACGG-3' digestion product upon incubation with codon-optimized RNase U2 for 30 minutes



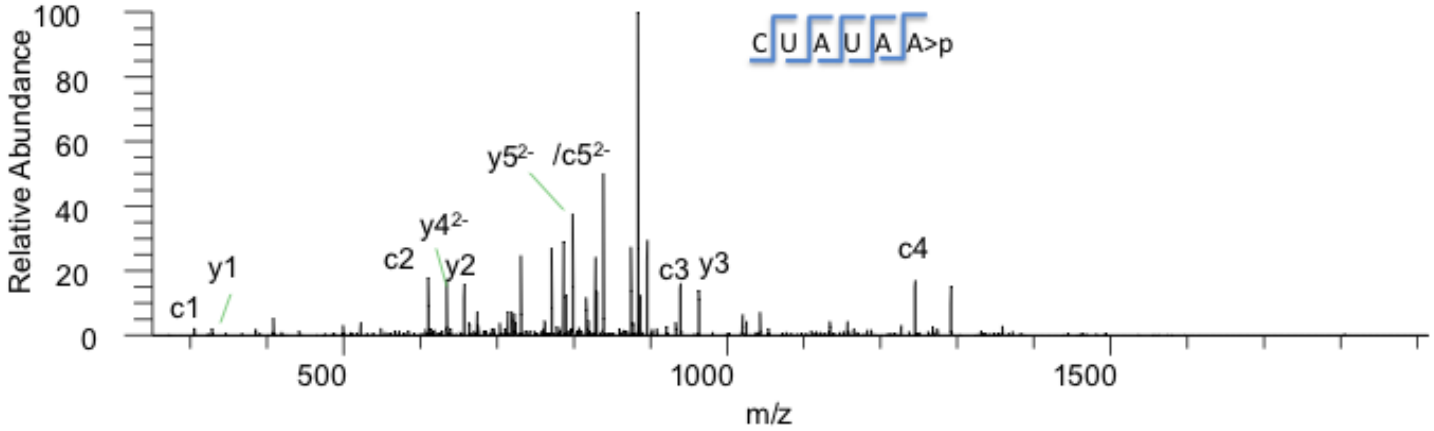
Supplemental Figure S14. CID mass spectrum with product ion assignments of the 5’-CUAUAACGG-3’ digestion product upon incubation with codon-optimized RNase U2 for 30 minutes



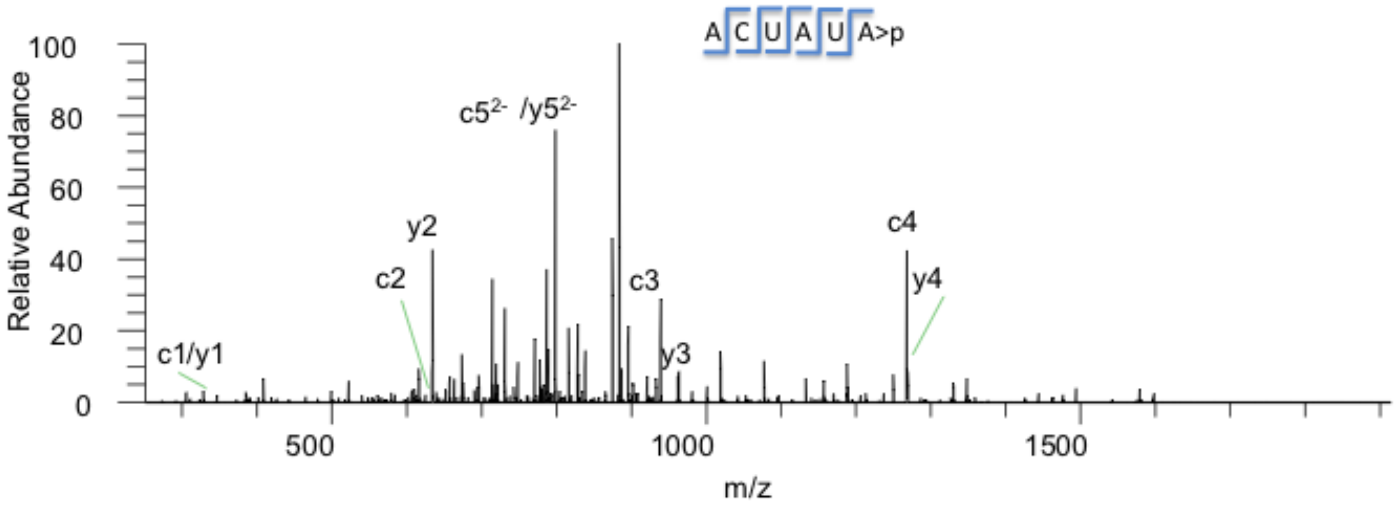
Supplemental Figure S15. CID mass spectrum with product ion assignments of the 5'-ACUAUAACGG-3' digestion product upon incubation with codon-optimized RNase U2 for 30 minutes



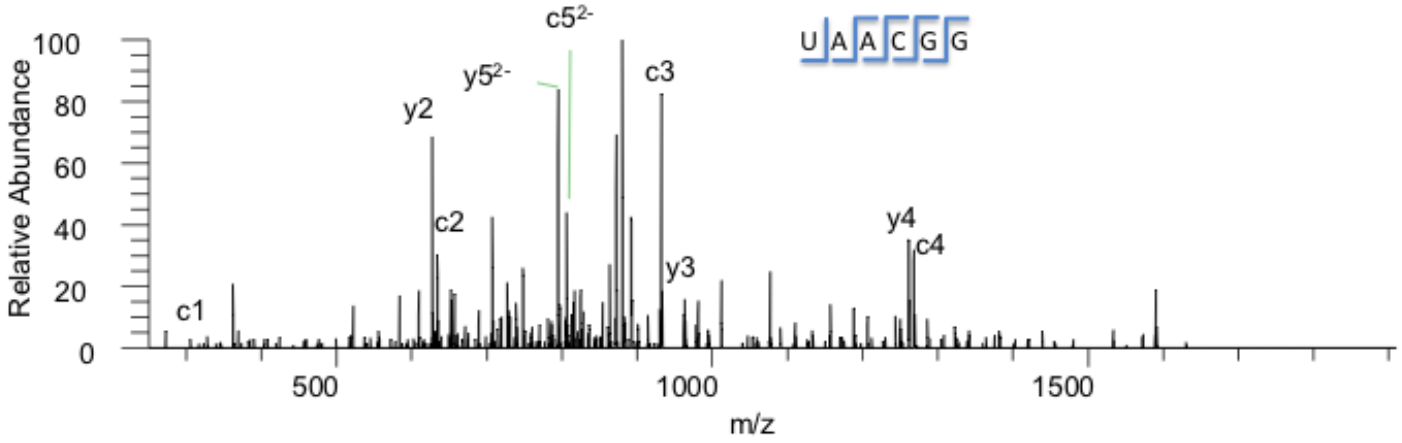
Supplemental Figure S16. CID mass spectrum with product ion assignments of the 5’-UAACUA-3’ digestion product upon incubation with codon-optimized RNase U2 for 30 minutes



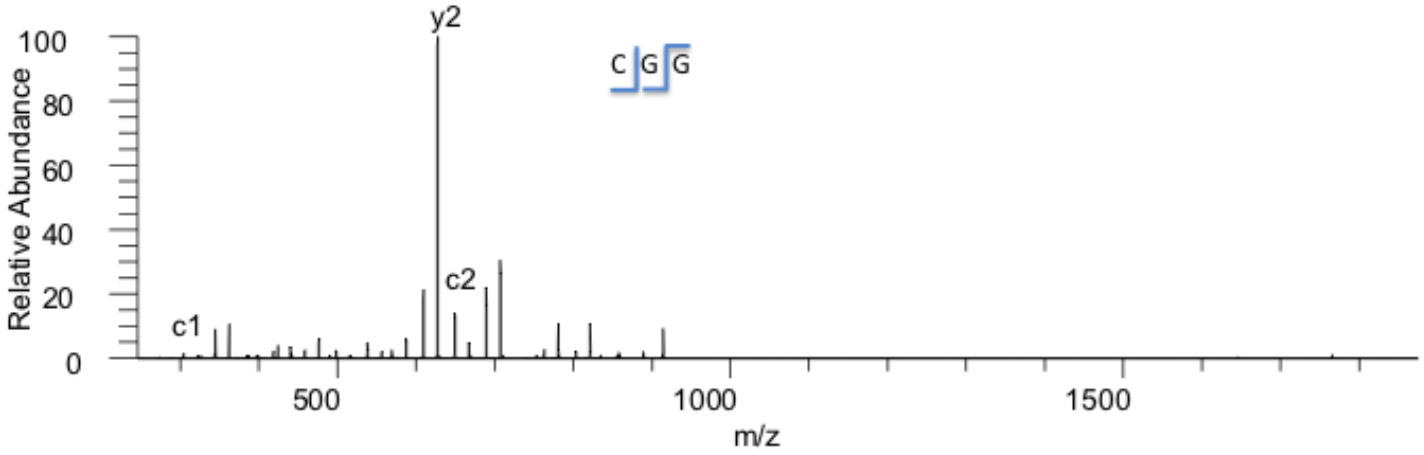
Supplemental Figure S17. CID mass spectrum with product ion assignments of the 5'-CUAUAA>p3' digestion product upon incubation with codon-optimized RNase U2 for 30 minutes



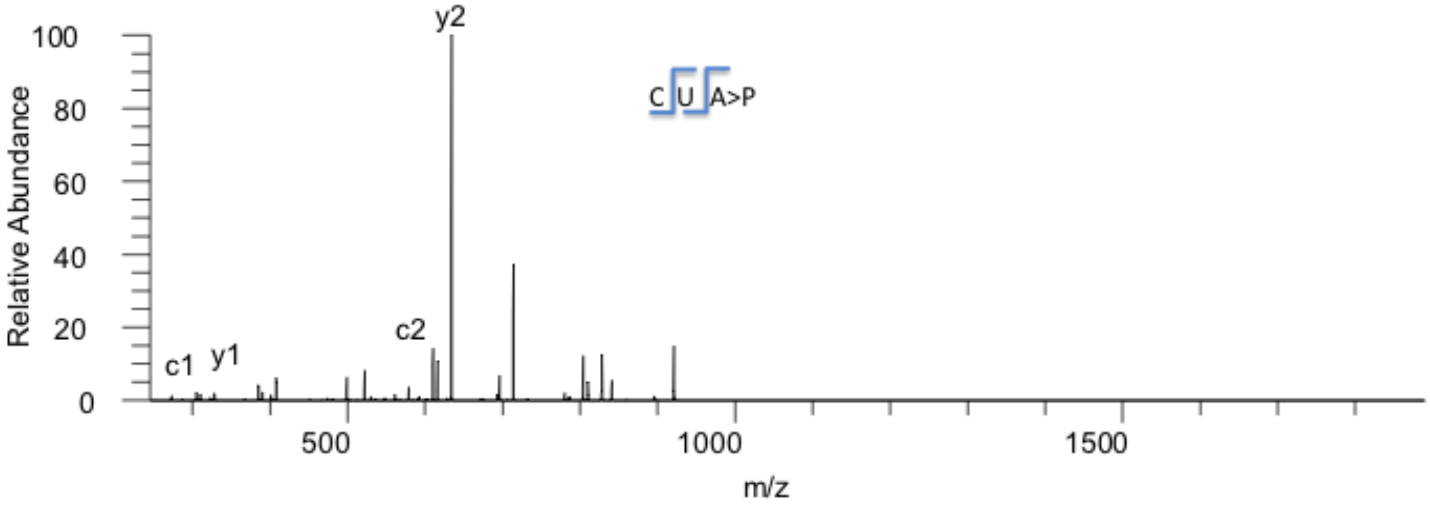
Supplemental Figure S18. CID mass spectrum with product ion assignments of the 5'-ACUAUA>p-3' digestion product upon incubation with codon-optimized RNase U2 for 30 minutes



Supplemental Figure S19. CID mass spectrum with product ion assignments of the 5’-UAACGG-3’ digestion product upon incubation with codon-optimized RNase U2 for 30 minutes



Supplemental Figure S20. CID mass spectrum with product ion assignments of the 5’-CGG-3’ digestion product upon incubation with codon-optimized RNase U2 for 30 minutes



Supplemental Figure S21. CID mass spectrum with product ion assignments of the 5’-CUA>p-3’ digestion product upon incubation with codon-optimized RNase U2 for 30 minutes

A

5'-GCGGAUUUA[m^{2}G]CUCAGDDGGGAGAGC[m$^2_2$G]CCAGA[Cm]U[Gm]AA[yW]A[Ψ][m^{5}C]UGGAG[m^{7}G]UC[m^{5}C]UGUGT[Ψ]CG[m^{1}A]UCCACAGAAUUCGCACCA-3'

B

5'-GCGGAUUUA[m^{2}G]CUCAGDDGGGAGAGC[m$^2_2$G]CCAGA[Cm]U[Gm]AA[yW]A[Ψ][m^{5}C]UGGAG[m^{7}G]UC[m^{5}C]UGUGT[Ψ]CG[m^{1}A]UCCACAGAAUUCGCACCA-3'

C

5'-GCGGAUUUA[m^{2}G]CUCAGDDGGGAGAGC[m$^2_2$G]CCAGA[Cm]U[Gm]AA[yW]A[Ψ][m^{5}C]UGGAG[m^{7}G]UC[m^{5}C]UGUGT[Ψ]CG[m^{1}A]UCCACAGAAUUCGCACCA-3'

D

5'-GCGGAUUUA[m^{2}G]CUCAGDDGGGAGAGC[m$^2_2$G]CCAGA[Cm]U[Gm]AA[yW]A[Ψ][m^{5}C]UGGAG[m^{7}G]UC[m^{5}C]UGUGT[Ψ]CG[m^{1}A]UCCACAGAAUUCGCACCA-3'

Supplemental Figure S22. Digestion products of *S. cerevisiae* tRNA-Phe with 5 ng E49A mutant incubated as described. **A.** Linear temperature gradient starting at 28 °C and ending at 65 °C in 7 minutes. **B.** Linear temperature gradient starting at 37 °C and ending at 65 °C over 5 minutes. **C.** Linear temperature gradient starting at 65 °C and ending at 45 °C over 30 minutes. **D.** Incubation at 65 °C, 45 °C and 37 °C at 5 minute intervals. Overall sequence coverage of tRNA from LC-MS/MS analysis is underlined.

Supplemental Table S1. Enzyme amounts and resulting tRNA sequence coverage for temperature ramp experiment. The enzyme used was the E49 mutant. Sequence coverage determined by LC-MS/MS. A linear temperature gradient starting at 28 °C and ending at 65 °C in 7 minutes provided sufficient incubation conditions for nearly complete sequence coverage of 5 µg of *S. cerevisiae* $tRNA^{Phe}$, with optimal results obtained when 1 ng of enzyme was used.

Amount of Enzyme (ng)	Sequence Coverage
0.1	89%
0.2	87%
0.5	96%
1.0	100%
1.5	83%
2.0	89%